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Effect of cobalt nano-particles on serum biochemical and histopathological changes in liver and kidney of lambs

Seyed Morteza Ghoreishi[1], Hossein Najafzadeh[2*],Babak Mohammadian[3], Eisa Rahimi[4], Mohammad Reza Afzalzadeh[5],Mohammad Kazemi Varnamkhasti[5], Hadi Ganjeali Darani[5]

[1] Student of Large Animal Internal Medicine, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
[2] Department of Pharmacology & Toxicology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
[3] Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
[4] Department of Chemistry, Payame Noor University, Tehran, Iran
[5] DVM, Student of, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran

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Abstract

Cobalt is an essential co-factor in red blood cell production and function and its deficiency may produce clinical signs in sheep. Thus, present study was designed to evaluate the effect of cobalt nano-particles on serum biochemical factors and histopathological changes in liver and kidnies of lambs. Study was carried out in 3 groups of lambs (4 lambs per group). One group of lambs was kept as control group. Second and third group respectively received cobalt nano-particles and conventional cobalt chloride suspension daily for a period 25 day. Blood sample and then serum was collected before and at the end of study. Activity of ALT, AST, ALP and level of BUN, creatinine and vitamin B12 were measured in serum of lambs. Tissue sections of liver and kidney were stained with hematoxylin and eosin and examined by light microscopy. Activity of ALT, BUN and vitamin B12 was significantly increased by cobalt nano-particles and conventional cobalt chloride. Fatty change of hepatocytes occured by conventional cobalt and granulomatous hepatitis, focal necrosis of hepatocytes and degeneration of hepatocytes by the nano cobalt was identified in liver. There were not any significant lesions and alteration in the kidnies of treated groups. Thus, cobalt nano-particles have similar effect to conventional cobalt for using in sheep with cobalt deficiency.

Keywords: Cobalt nano-particles, hepatotoxicity, renal toxicity, lamb

*corresponding author: Hossein Najafzadeh
Email: najafzadeh@scu.ac.ir

Introduction

The trace element; cobalt is a dietary essential element for ruminants, allowing synthesis of Vitamin B12 by rumen microorganisms (Tiffany, et al 2003). Vitamin B12 is a cofactor for two enzymes; methyl malonyl-Co A mutase that catalyzes the interconversion of methyl malonyl-Co A to succinyle –Co A, an important step in glucogenesis and methionine synthase that acts to remethylate homocysteine in the terminal step of methionine synthesis (Simonsen *et al*., 2012). In ruminants, Co-induced Vitamin B_{12} deficiency disturbs normal energy and protein metabolism and their demand has to be ensured by continuous adequate supply of dietary Co (Kennedy *et al*.,1990; Larry, 2005). Sheep tend to be extremely susceptible to Co deficiency because their Co requirement is about twice than of cattle (Grace et al., 2000). Vitamin B12 deficiency in sheep is clinically manifested as anaemia, inappetence, weight loss, poor production, lacrimation, photosensitivity, alopecia and immune deficiency (Vellema *et al*., 1997). Vitamin B12 deficiency has been shown to cause depletion of intracellular folate concentration in sheep liver (Smith *et al*., 1973). Cobalt deficiency has also long been incriminated as the cause of fatty hepatic degeneration that has been termed ovine white liver disease (Vellema *et al*., 1996) or chronic hepatitis (Kennedy *et al*., 1997).

By rapid development of nanotechnology, the usage of nanoparticles to replace normal-scale particles has been rapidly increased (Popov *et al*., 2005). Metal oxide nanoparticles are among the highest nanomaterials production. But some toxicological studies have shown that when nanoparticles entered into the body through several distinct routes including inhalation, ingestion, and dermal penetration could elicit toxicological effects at different levels of biological systems (Jeng and Swanson, 2006; Sayes *et al*., 2007). Therefore investigation of their effects on the body health is necessary. The aim of this study was to evaluate the oral toxicity of nanoscale cobalt in lambs. Additionally, the effects of particles on some serum biochemical factors, and histopathological changes in the liver and kidnies were also investigated.

Materials and methods

Cobalt nanoparticle powder was purchased from US Research Nanomaterials, Inc. Houston, USA. The administrated particles were suspended in normal saline. The size of the nano particle was determined as 50 nm diameters, by transmission electron microscopy (TEM, XRD, JEM200CX).

The experiment was begun in July 2011 and finished in September 2011; using 12 male lambs (aged 5 - 6 months and weighed 18-20 kg). The lambs were allowed free access to diet and water. Study was carried out in 3 groups of lambs (4 lambs each group). One group of lambs was kept as control group. Second and third group received cobalt nano-particles and conventional cobalt chloride suspension daily for 25 days respectively. After one week acclimation, the suspension of cobalt nano particle was orally administrated to lambs by dose of 10mg/kg body weight. Blood was collected before and at the end of the study and serum was separated by centrifugation. These samples were stored at -20 C until analysis. The biochemical levels of alanine aminotransferas (ALT), aspartate aminotransferase(AST), alkaline phosphatase (ALP), blood urea nitrogen (BUN), creatinine (CR), were assayed by an automatic biochemical analyzer (Cobas Mira Plus , Roch Diagnostics , Germany). Vitamin B_{12} was evaluation using Radio assay kit in (purchased from Simul TRAC- SNB- Biochemicals, Germany) was used for simultaneous quantitative determination of Vitamin B_{12} in serum.

The animals were sacrificed after 25 days. The liver and kidnies were collected, while all tissues were fixed in 10% buffered formalin for histopathological examination. Then, tissues were embedded into the paraffin,

sectioned for 5 μm thickness, and mounted on the glass microscope slides using standard histopathological techniques. The sections were stained with hematoxylin-eosin and examined by light microscopy.

All data were subjected to statistical analysis including the calculation of the mean and standard error by using SPSS version 16. $p<0.05$ was determind as significancy of results.

Results

All animals were alive at the end of the study. The serum vitamin B_{12} level and effect of oral administration of cobalt nanoparticles on the serum biochemical levels of lambs are shown in Table 1 and Figures 1-3. Increase of vitamin B_{12} level was observed before and after of oral exposure to cobalt, but a sharp elevation was seen by use of nanoparticles ($p<0.05$). The mean concentration of vitamin B_{12} in serum at the end of the experiment was 1553 pmol/l in nanoparticles administrated group whereas before nanoparticles supplementation, the mean value was 554.33 pmol/l (Table 1).

The results indicated that activity of BUN was significantly increased after cobalt nanoparticle administration ($p= 0.005$). The level of the serum ALT was significantly increased after administration of vitamin B_{12} nanoparticles ($p=0.003$). The level of other factors did not change significantly (Table 1 and Fig. 2).

Histopathological findings of the liver and kidnies are illustrated in Figures 4 and 5. Liver in the conventional cobalt group showed fatty change of hepatocytes. Liver in the nano cobalt group showed granulomatous hepatitis, focal necrosis of hepatocytes and degeneration of hepatocytes (Fig. 4). No significant lesions were found in the kidnies of treated groups (Fig. 5).

Discussion

The animals had normal range of serum vitamin B12 at the beginning of the study. Marginal reference range of serum vitamin B12 concentration is 336-499 pmol/l (Gruner *et al*., 2004). Serum level of vitamin B_{12} was significantly increased by oral administration of nano cobalt particles in lambs in the present study. Also nano cobalt significantly elevated serum VitB12 concentrations in comparison with cobalt chloride. This result was similar to the research conducted by Oberd¨orster *et al*. (1994) that showed nanoscale particles had large specific surface area. It was found that their biological effects were mainly dependent on their surface area rather than particle mass (Oberd¨orster *et al*., 1994). Cobalt deficiency in sheep is important (Kennedy *et al*., 1997). Studies also indicate the necessity of increasing the amount of dietary cobalt for growing ruminants up to a level of 300-500 μg/kg dry matters for optimum microbial activity, fermentation and Vitamin B_{12} synthesis (Singh and Chhabara, 1995). The increase of cobalt intake may improve ruminal fermentation related to alteration in the ruminal microbial population, specifically the cellulolytic bacteria (Scholljegerdes *et al*., 2010).

The blood biochemical tests are frequently used in the diagnosis of liver and kidney diseases. They are also widely used to monitor the response to the exogenous toxic exposure. In ruminants AST is often tested along with LDH and ALP to evaluate damaged or diseased liver. In liver dysfunction, the levels of above enzymes (AST, LDHR, ALP) will rise. ALT is considered to be liver specific in cat and dog but there is little hepatic ALT activity in sheep. The value of serum ALT as an indicator of hepatocellular necrosis has been clearly shown, especially in dogs and cats, but to a much lesser extent in horses, cattle, swine, sheep, and goats (Spano *et al*., 1983; Turgut *et al*., 1997). Therefore, in this study the activity of AST did not elevate significantly. In addition, the histopathological findings confirmed mild liver toxicity by nano cobalt (Fig. 4). Histopathological examination of the liver revealed granulomatous hepatitis, necrosis of hepatocytes in the nano cobalt

Table 1. Mean (± SE.) of some serum factors in lambs with or without nano particles and conventional cobalt treatment.

	Creatinine (mg/dl)		Alkaline phosphatase (iu/l)		Vitamin B12 (pmol/l)	
Day	**0**	**25**	**0**	**25**	**0**	**25**
Nanocobalt	1.05 ± 0.1041	1.775±0.2016	498.33± 71.354	197±55.073	554.33± 52.6	1553±154.037
Cobalt	1.225± 0.925	1.825±0.3683	153± 38.837	104±16.083	755.67±129.633	1202.33±119.066
Control	0.925± 0.075	0.9 ±0.1732	203.5± 89.5	156.5±21.5	-	-

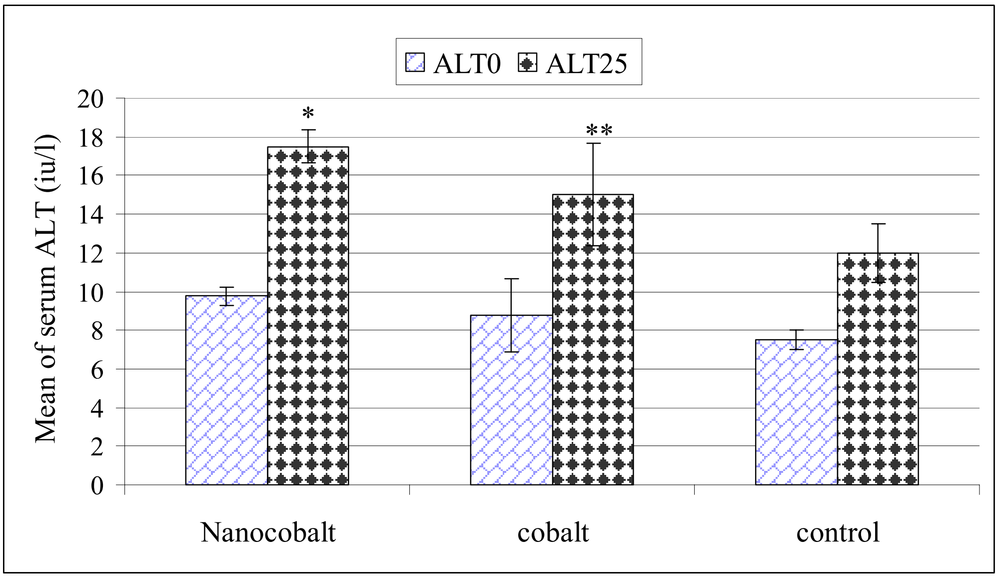


Figure 1. Mean ± SEM of serum ALT in lambs. * and ** show significant difference between first day and 25th day of sampling. (*p*<0.05 and n=4).

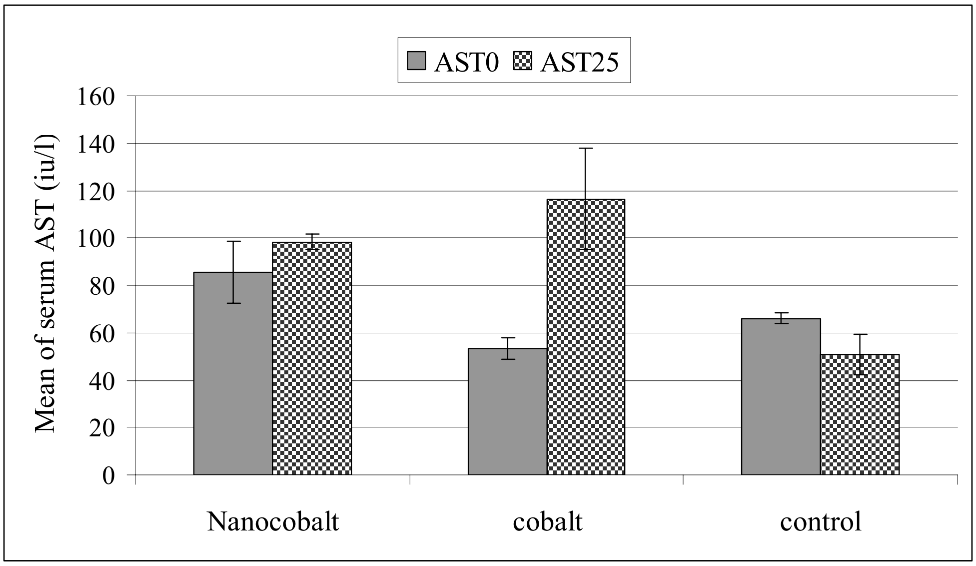


Figure 2. Mean ± SEM of serum AST in lambs.

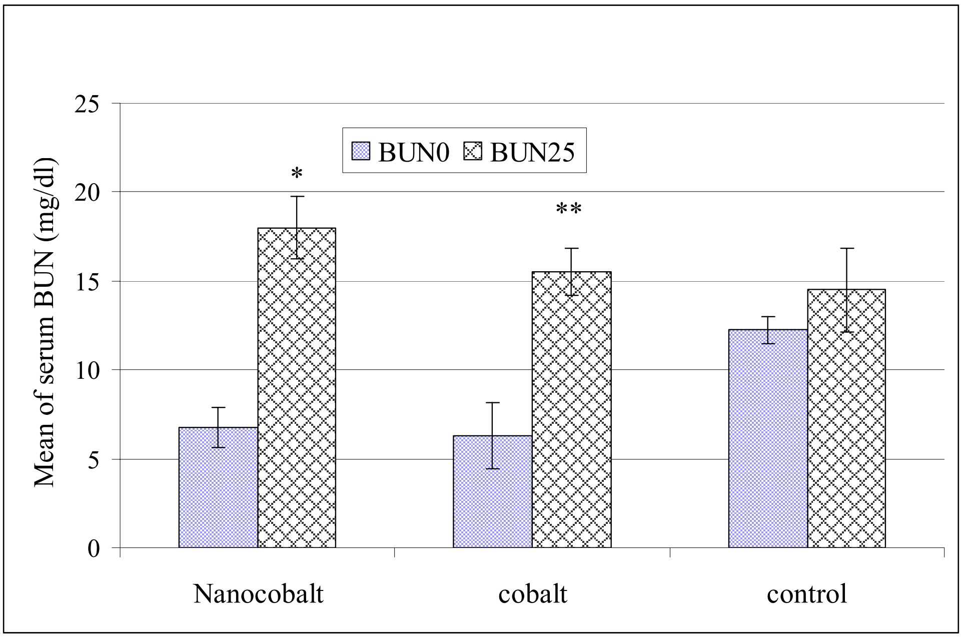


Figure 3. Mean ± SEM of BUN in lambs. * and ** show significant difference between first day and 25th day of sampling. (*p*<0.05 and n=4).

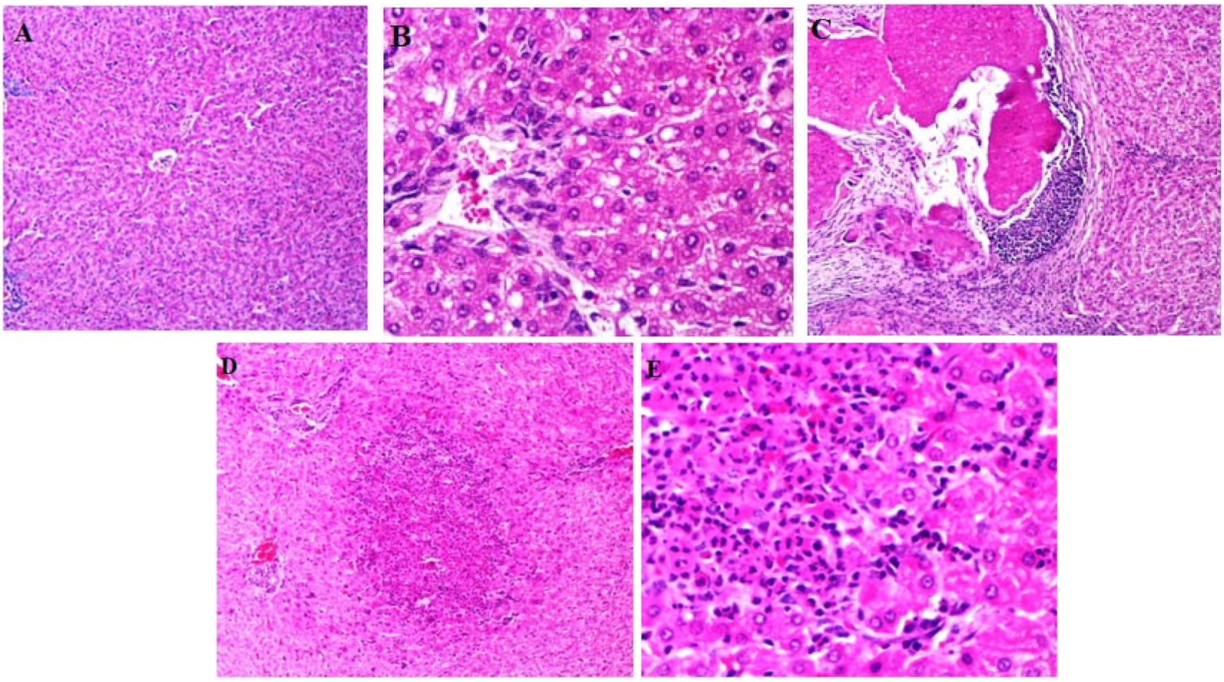


Figure 4. Liver tissue from lambs of control group, lambs exposed to nano cobalt and conventional cobalt (A) Liver in the control group, showing normal structure. Hematoxylin and eosin, ×10. (B) Liver in the chloride cobalt group shows fatty change of hepatocytes Hematoxylin and eosin, ×40. (C) Liver in the nano cobalt group shows granulomatous hepatitis, Hematoxylin and eosin, ×10. (D) Liver in the nano cobalt group shows focal necrosis of hepatocytes Hematoxylin and eosin, ×10. (E) Liver in nano cobalt group shows degeneration of hepatocytes and neutrophils cells Hematoxylin and eosin, ×40.

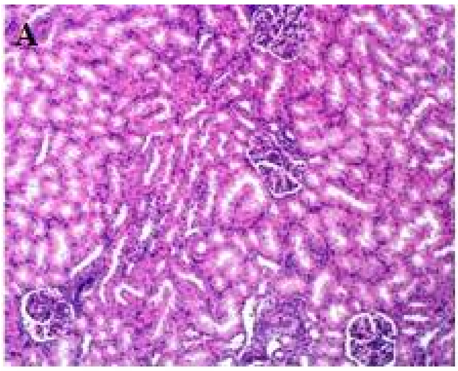


Figure 5. Histopathological findings of kidney of lambs exposed to nano cobalt and conventional cobalt at dose of 10mg/kg body weight on 25days oral administration. There are no significant lesions in kidney of nano particles group. (H&E), (10x).

administered lambs and fatty change of hepatocytes in the cobalt chloride administered group. Almost similar hepatotoxicity has been reported by Garoui *et al.,* (2011) in rats by cobalt chloride. The histopathological findings demonstrated that the nano cobalt oral exposure could lead to more severe liver damage than the cobalt chloride, Although the serum indicators did not show obvious changes. Cobalt is accumulated primarily in liver, kidney, pancreas, and heart. Cobalt metal and salts are induced oxidative DNA damage by reactive oxygen species, perhaps combined with inhibition of DNA repair (Simonsen *et al*., 2012).

The blood BUN and creatinine are good indicators for renal function. Renal dysfunction leads to increased BUN and creatinine levels. Thus, the significant increased in BUN level in the treated groups in the present study may indicate the renal dysfunction but is not exactly an important factor. Renal toxicity caused by cobalt chloride has been reported by Naura and Sharma (2009). They suggested that higher accumulation of cobalt in kidney may induce higher oxidative stress in this organ and nephrotoxicity (Naura and Sharma 2009). The histopathological finding in the present study demonstrated that the nano cobalt oral exposure could not produce renal damage.

Combined biochemical evaluation and histopathological results in the present study, indicate that the oral administration of 10mg/kg nano cobalt may cause toxic effects in liver but not in kidney. The effect of nano cobalt particle on lamb's liver and kidnies were similar to conventional cobalt. However, the use of nano cobalt may have more beneficial effect in cobalt-deficiency.

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اثر ذرات نانو کبالت بر روی تغییرات بیوشیمیایی سرم و آسیب شناسی بافتی در کبد و کلیه بره ها

سید مرتضی قریشی[۱]، حسین نجف زاده[۲*]، بابک محمدیان[۳]، عیسی رحیمی[۴]، محمد رضا افضل زاده[۵]، محمد کاظمی ورنا مخواستی[۵]، هادی گنجعلی دارانی[۵]

[۱] دانشجوی دکتری تخصصی بیماریهای داخلی دامهای بزرگ، دانشکده دامپزشکی دانشگاه شهید چمران اهواز، اهواز، ایران
[۲] استاد گروه فارماکولوژی و سم شناسی، دانشکده دامپزشکی دانشگاه شهید چمران اهواز، اهواز، ایران
[۳] دانشیار گروه پاتوبیولوژی، دانشکده دامپزشکی دانشگاه شهید چمران اهواز، اهواز، ایران
[۴] گروه شیمی، دانشگاه پیام نورتهران، تهران، ایران
[۵] دانشجوی دکتری عمومی دامپزشکی، دانشکده دامپزشکی دانشگاه شهید چمران اهواز، اهواز، ایران

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چکیده

کبالت ازفاکتورهای ضروری در تولید و عملکرد گلبولهای قرمز بوده و کمبودش در گوسفند می‌توانـد تولیـد نشـانه‌هـای بـالینی نمایـد. بنابراین مطالعه حاضر جهت بررسی اثرات ذرات نانو کبالت بر روی فاکتورهای بیوشیمیایی سرم و تغییرات آسـیب شناسـی بـافتی در کبـد و کلیه بره طراحی گردید. مطالعه در سه گروه که هر گروه متشکل از چهار بره می‌بود طراحی شد. گروه اول به عنوان گروه کنترل، گـروه دوم و سوم بترتیب به عنوان دریافت کننده‌های ذرات نانوکبالت و سوسپانسیون کلرید کبالت معمولی به مدت بیست و پنج روز در نظر گرفته شد. نمونه خون و سپس سرم قبل از شروع مطالعه و درپایان مطالعه اخذ گردید. فعالیت آنزیمهای ALT، AST، ALP، سطحBUN، کراتینین و ویتامینB_{12} در سرم بره‌ها اندازه گیری شد. مقاطعی از کبد و کلیه توسط هماتوکسیلین- ائوزین رنگ آمیزی و سـپس توسـط میکروسـکوپ نوری مورد بررسی قرار گرفت. فعالیت ALT ، سطح BUN و ویتامینB_{12} در گروه‌هـای دریافـت کننـده ذرات نانوکبالـت و کلریـد کبالـت معمولی افزایش معناداری را نشان داد. تغییرات چربی در سلولهای کبد بوسیله کبالت معمولی و التهاب گرانولوماتوز کبـدی، نکروزموضـعی و دژنره شدن سلولهای کبدی بوسیله نانوکبالت در کبد تشخیص داده شد. در گروهـای درمـانی ضـایعات و یـا تغییـرات معنـادارکلیوی وجـود نداشت. بنابراین در موارد کمبود کبالت در گوسفند ذرات نانو کبالت جهت استفاده اثرات مشابه‌ای با کبالت معمولی دارند.

واژگان کلیدی: ذرات نانو کبالت،سمیت کبدی، سمیت کلیوی، بره